



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 9/113, 39/02, 39/05, 39/08, 39/116	A1	(11) International Publication Number: WO 98/27964 (43) International Publication Date: 2 July 1998 (02.07.98)
(21) International Application Number: PCT/AU97/00871 (22) International Filing Date: 22 December 1997 (22.12.97) (30) Priority Data: 9626865.1 24 December 1996 (24.12.96) GB (71) Applicant (for all designated States except US): FORT DODGE AUSTRALIA PTY. LIMITED [AU/AU]; 23 Victoria Avenue, Castle Hill, NSW 2154 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only): BUCHTA, Richard [AU/AU]; 23 Gum Blossom Drive, Westleigh, NSW 2120 (AU). SCHWARTZKOFF, Christopher, Leigh [AU/AU]; 15 Holmes Street, Turrumurra, NSW 2075 (AU). LEHRBACH, Philip, Ralph [AU/AU]; 5 Fiona Avenue, Wahroonga, NSW 2076 (AU). (74) Agent: SPRUSON & FERGUSON; G.P.O. Box 3898, Sydney, NSW 2001 (AU).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: VETERINARY VACCINES (57) Abstract <p>Stable vaccine compositions allow safe and efficacious use of an oil emulsion vaccine for the initial single dose vaccination of previously unvaccinated animals and/or the subsequent annual booster vaccination. The vaccine compositions have a viscosity of 100m Pas or less and comprise multi-phase emulsions including an oil phase acting as adjuvant and preservative and an aqueous phase including one or more antigens selected from diseases of cattle or sheep. The oily adjuvant is acceptable for veterinary purposes and comprises a synthetic hydrocarbon being between 40 % and 60 % by weight of the emulsion and an emulsifier, selected so that the inversion point of the resulting emulsion is between 25 °C and 45 °C, and being between 2 % and 10 % by weight of the emulsion.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

- 1 -

VETERINARY VACCINES

5 The present invention relates to novel vaccine compositions for parenteral administration, methods for their use and to processes for their preparation.

10 Bacterial and viral diseases of cattle, sheep and pigs, such as Clostridial diseases, cause considerable economic damage in the agriculture industry. Vaccination is therefore a very important means of controlling these diseases.

15 Currently available vaccines comprise antigens absorbed onto alkali earth metal salts such as aluminium phosphate or aluminium hydroxide and water in oil emulsion vaccines. The animals have to be mustered each year for an annual vaccination, however, these vaccines have to be administered to naive animals, i.e. animals which have not been previously vaccinated, in a two stage or two dose regime consisting of an initial dose and a second "booster" dose a few weeks later which raises the antibody level to a level which sustains protection for a year. This means that the animals must either be kept corralled for the period until the booster dose is given or they have to be mustered again. This is time consuming and expensive and such methods of vaccination are therefore undesirable.

20 The aluminium based vaccines have been found to have relatively short durations of protection while water-in-oil emulsion vaccines have longer durations of protection, but have been found to be unsuitable for use because they cause unacceptable lesions at the injection sites of the animals ('Experimental Clostridial Oil Emulsion Vaccines' Thomson R.O. and Batty I., Bull. Off. int. Epiz. 1967 67 (11-12) 1569-1581; 'The Immunogenicity of a multicomponent Clostridial Oil Emulsion Vaccine in sheep' Thomson *et al*, The Veterinary Record, 26 July 1969). In 1976 Jansen *et. al.* reported the immune response of *Clostridium botulinum* C and D toxoids in a water-in-oil emulsion vaccine and noted that the two-stage aluminium based vaccine was not boosted by the second dose to the same extent as the water in oil compositions (Jansen, B C, Knoetze, P C & Visser, F; Onderstepoort J Vet Res, 43(4) 165-174 (1976)). However, the water in oil compositions gave an undesirable granulomatous swelling resulting from subcutaneous injection of the vaccine in a large percentage of animals which is a severe disadvantage for the vaccine's routine use in commercial cattle.

- 2 -

WO 91/00106 discloses multi-phase emulsions suitable for administering active substances or antigens by injection of the water in oil in water type. These emulsions are produced from pharmaceutically acceptable emulsifiers which when dissolved in an injectable oil, form a homogeneous clear phase and have inversion points
5 approaching the temperature of human or animal bodies. The oils contained in the emulsions include mineral, vegetable or animal oils, and synthetic hydrocarbons. It was observed that these vaccines were well tolerated in pigs and did not cause any local reactions, abscesses or necroses. However, no data were provided regarding the level and duration of the immune responses.

10 The applicants have found that adjuvants containing synthetic hydrocarbons are particularly suitable for vaccines for the prevention of diseases of sheep and cattle and for the majority of diseases provide an effective immunity for up to a year or more following a single injection or dose including in naive sheep and cattle. Therefore,
15 the present invention addresses the problems associated with known vaccines, providing a level of effective immune response in naive cattle or sheep for the period of approximately one year or more following a single injection or dose of vaccine.

20 Thus according to the present invention there is provided a vaccine composition which is an injectable multi-phase emulsion of the water in oil in water type having a viscosity of 100mPas or less, comprising:

a) an oily adjuvant acceptable for veterinary purposes, comprising:

i) a synthetic hydrocarbon being between 40% and 60% by weight of the emulsion and

25 ii) an emulsifier selected so that the inversion point of the resulting emulsion is between 25°C and 45°C and being between 2% and 10% by weight of the emulsion; and

b) an aqueous phase comprising one or more antigens selected from diseases of cattle and sheep.

30 The synthetic hydrocarbon, is preferably one which is liquid at 4°C and has a viscosity lower than 100mPas at 25°C. Preferred synthetic hydrocarbons include polyisobutenes and polyisoprenes. Polyisobutylene is particularly preferred.

35 Antigens suitable for use in the compositions of the present invention include antigens derived from bacterial and viral pathogens of sheep and cattle. Preferred bacterial antigens include Clostridial antigens such as Clostridium botulinum C and

- 3 -

D, *Clostridium perfringens* type A, B, C and D, *Clostridium septicum*, *Clostridium tetani*, *Clostridium chauvoei*, *Clostridium novyi* type B, *Clostridium sordellii*, *Clostridium haemolyticum*; *Leptospira* antigens, for example, *Leptospira interrogans* such as *Leptospira hardjo*, *Leptospira pomona*, *Leptospira copenhageni*, *Leptospira*
5 *zanoni*, *Leptospira tarassovi*; *Pasteurella* antigens such as *Pasteurella multocida* and *Pasteurella haemolytica*; *Corynebacterium* antigens such as *Corynebacterium pseudotuberculosis*, *Corynebacterium renale*, *Corynebacterium cystitis*, *Corynebacterium pilosum* and *Corynebacterium bovis*; and *Haemophilus* antigens such as *Haemophilus somnus* and *Haemophilus pleuropneumoniae*; *Dichelobacter*
10 *nodosus pilus*; *Mycoplasma* antigens such as *Mycoplasma agalactiae*, and *Mycoplasma ovipneumoniae*. Preferred viral antigens include Bovine Viral Diarrhoea (BVD) antigens, Bovine Rhinotracheitis Virus (IBR) antigens, Parainfluenza-3 antigens, Respiratory Syncytial Virus (RSV) antigens and Bovine Ephemeral Fever (BEF) antigens.

15 Particularly preferred embodiments of the invention include botulinum C and/or D toxins as antigens. Suitable antigens include those which are useful in the treatment of diseases such as Lamb dysentery, Pulpy Kidney disease (enterotoxemia), Malignant Oedema (blood poisoning), Tetanus, Blackleg disease, Black disease,
20 caseous lymphadenitis, ovine foot rot, pasteurellosis and botulism.

Embodiments of the invention include those which are multivalent vaccines, i.e. vaccines which provide protection against a number of different diseases by incorporating a number of different antigens e.g. the vaccine may contain any number
25 of antigens selected from the list provided above. It is particularly useful to provide a multivalent vaccine, i.e. one which provide adequate immune response to a number of pathogens to increase the range of protection provided by the vaccine. It is particularly difficult to provide multivalent vaccines because it is necessary to provide a vaccine which induces an adequate antigenic response to all the micro-
30 organisms of interest. Thus the threshold antibody responses are described in compendial standards (e.g. Australian Therapeutic Goods order No. 30; British Pharmacopoeia; European Pharmacopoeia and United States Code of Federal regulation). Where compendial standards do not exist (e.g. for *Corynebacterium pseudotuberculosis*) recognised thresholds based on protection from challenge are
35 accepted.

- 4 -

Vaccines provided by the invention include, but are not limited to, 3, 4, 5 and 6 valent vaccines. They may provide protection against a number of diseases of the same type, e.g. the vaccines may contain a number of similar antigens. Alternatively the vaccines may provide protection against a number of diseases caused by different types of micro-organism.

Preferred embodiments of this type are vaccines comprising at least two types of antigen, each one being active against any one of the following: *Clostridium botulinum*, *Clostridium perfringens*; *Clostridium novyi*; *Clostridium chauvoei*; *Clostridium septicum*, *Clostridium tetani* and *Corynebacterium pseudotuberculosis*. Particularly preferred embodiments are vaccines comprising an antigen to all the diseases listed above.

Particular embodiments are vaccines comprising at least two of the following types of antigen: *Clostridium botulinum* C toxin, *Clostridium botulinum* D toxin, *Clostridium perfringens* D toxoid; *Clostridium novyi* B toxoid; *Clostridium chauvoei* anaculture; *Clostridium septicum* toxoid, *Clostridium tetani* toxoid and *Corynebacterium pseudotuberculosis*. Particularly preferred embodiments are vaccines comprising all the antigens listed.

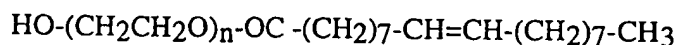
The vaccines of the invention may suitably also include mineral supplements such as selenium or cobalt (vitamin B12) and/or anthelmintics such as avermectins (e.g. abamectin) and milbemycins (e.g. moxidectin).

The emulsifiers included in the vaccine are non-ionic products such as fatty acid esters or ethers of sugars and polyols, e.g. sorbitol, mannitol, saccharose, glucose and glycerol, and hydrophilic derivatives of such esters, e.g. alcohol, ether-oxide, carboxylic acid, amine and amide derivatives. Lecithins and fatty acids and/or alcohols condensed with ethylene and/or propylene oxide are examples of suitable emulsifiers. The fatty chains of the emulsifiers normally have between 8 and 22 carbons atoms and preferably between 14 and 20 carbon atoms. Preferred fatty acids are oleic, stearic and ricinoleic acids. Liquid emulsifiers are preferred.

More specifically preferred emulsifiers include fatty acid esters of sorbitol, mannitol, glycerol, polyethylene and/or propylene oxides, glycerophospholipids or a mixture of any two or more thereof. Emulsifiers of particular interest in the present invention

- 5 -

are mannide oleate (or mannitol monooleate ester) and PEG oleates, e.g. PEG-10-oleate. Particularly preferred emulsifiers are oleic esters of the following formula:



5 wherein n is an integer from 1 to 18.

Co-adjuvants may optionally be included in the vaccines of the present invention. The antigens may be in the form of toxoids or cell antigens but if cell antigens are used a co-adjuvant may be required such as a saponin, e.g. quil A, or cytokines such as Interleukin-1, 2 and/or 4 or muramyl dipeptide. Further emulsifiers such as dioctyl
10 decyl ammonium bromide (DDA) may also be included in the vaccines if desired. The vaccine composition of the present invention may contain one or more antigens and one or more emulsifiers and/or one or more co-adjuvants.

15 Vaccines according to the present invention may be prepared by dissolving antigens in a suitable aqueous medium such as normal saline, stirring the resultant mixture and adding it to a suitable oil phase. The mixture is then stirred (e.g. at 200 to 600 rpm) and/or homogenised (e.g. at < 1000 psi) to the desired viscosity (<100 mPas) and conductivity (0.5 to 5.0 millisiemens at 20°C). Preservatives such as thiomersal may
20 optionally be included in the aqueous mixture prior to adding the antigens. This process is preferably carried out at about 20°C to about 25°C.

Surprisingly it has been found that the vaccines of the invention can provide a sustained and elevated immune response when administered to target animals, such as
25 cattle and sheep, in a single dose. The vaccines are suitable for administration to young and pregnant animals without adverse reaction. The vaccine compositions of the present invention are stable and may be stored for several months or even years without loss of antigenic potency.

30 The present invention will now be exemplified with reference to the following Examples by way of illustration only.

- 6 -

EXAMPLESEXAMPLE 1: PREPARATION AND EVALUATION OF A SINGLE DOSE BOTULINUM VACCINE

5

Vaccine compositions were prepared according to Table I below. Composition 1 was prepared by mixing botulinum toxoid antigens C and D with normal saline and thiomersal. This aqueous mixture was stirred at room temperature and added to a synthetic hydrocarbon oil phase (ISA207 obtained from SEPPIC SA, 75 Quai D-
 10 Orsay, 75007 Paris) equilibrated to room temperature (about 20°C to about 25°C). The mixture was stirred at 100 to 600 rpm at room temperature for 15 minutes (Conductivity 0.9 millisiemens, viscosity 30 CPS). Vaccine Composition 2 was prepared in the same way, except that an aluminium based adjuvant Tasgel[®] was used instead of ISA207.

15

Table I: Vaccine Compositions

Component	Composition	
	Amount (% v/v)	
	1	2
<i>Cl. botulinum</i> C toxoid concentrate	1.25	0.5
<i>Cl. botulinum</i> D toxoid concentrate	0.87	0.35
Water or Normal saline	41.78	68.15
Thiomersal	1	1
ISA207 [®]	55.1	-
Tasgel [®]	-	30

A group of fifty Murray Grey/Hereford cross cattle (approximately 12 month old
 20 naive cattle) were vaccinated by subcutaneous injection of the compositions described above and also with a further commercially available vaccine CSL Botulinum Vaccine Batch No 0708 15102 (also containing an aluminium based adjuvant). The vaccinations were performed according to the regimes described below in Table II.

- 7 -

Table II: Vaccination Regime

Vaccine	Dose Volume (mL)	No. Cattle	Vaccine Regime
(Control)	-	10	-
Composition 1	2	10	single vaccination Week 0
Composition 2	5	10	single vaccination Week 0
Composition 2	5	10	two vaccinations Weeks 0 and 5
CSL Botulinum Vaccine Batch No 0708 15102	5	10	single vaccination Week 0

Site reactions were recorded and found to be negligible for all groups. All site reactions had resolved seven to twelve weeks after vaccination. Overall the site reactions due to Composition 1 were slightly larger initially than the other vaccine formulations but these had also resolved completely by week 12. No adverse effects on the general condition of the cattle in any of the experimental groups following vaccination were reported. The mean site reactions are shown below in Table III.

Table III: Mean Site Reactions in Cattle

Vaccine	Mean Site Reactions (cm ³)			
	Week			
	5	7	12	18
(Control)	0	0	0	0
Composition 1	7.5	0.7	0	0
Composition 2	0	0	0	0
Composition 2	0	0	0	0
CSL Botulinum Vaccine	1.7	0.9	0	0

- 8 -

Serum samples were collected from the cattle over a 52 week period and mouse serum neutralisation titres on pooled group sera were carried out. *Cl. botulinum* C and D serum neutralisation (SN) titres are summarised below in Tables IV and V.

5 **Table IV: *Cl. botulinum* C SN Titres (units/mL)**

Vaccine	SN Titres (units/mL)						
	Week						
	0 ^a	5	7	12	18	26	52
(Control)	<4	<3.3	NT	NT	<1	<2.2	<2
Composition 1		<3.3	1.1-1.65	1.65-2.48	3.38-5.07	3.3-4.95	3.45
Composition 2		<3.3	NT	NT	<1	<2.2	NT
Composition 2		NT	>8.37	5.58-8.37	2.25-3.38	<2.2	<2
CSL Botulinum Vaccine		<3.3	NT	NT	<1	<2.2	NT

^a Prevaccination titres based on pooled serum from all groups.

NT = means not tested.

Table V: *Cl. botulinum* D SN Titres (units/mL)

10

Vaccine	SN Titres (units/mL)						
	Week						
	0 ^a	5	7	12	18	26	52
(Control)	<1.8	<1.8	NT	NT	<2	<2.2	<2
Composition 1		<1.8	<1.8	2.7	10.8-12.96	21.97-24.17	10.13-15.19
Composition 2		<1.8	NT	NT	<2	<2.2	NT
Composition 2		NT	>13.67	9.12-13.67	3.6-5.4	3.3-4.95	2-3
CSL Botulinum Vaccine		<1.8	<2	NT	<2	<2.2	NT

^a Prevaccination titres based on pooled serum from all groups

NT = means not tested

- 9 -

From the *Cl. botulinum* C and D SN titres it can be seen that a single vaccination of botulinum C and D vaccine of Composition 1 generated detectable SN titres by week 7 post-vaccination for *Cl. botulinum* C and week 12 post-vaccination for *Cl.*

5 *botulinum* D. Peak SN titres were observed for *Cl. botulinum* C and D at weeks 18 and 26 respectively. In contrast a single vaccination of a *Cl. botulinum* C and D vaccine formulated with Tasgel[®] (Composition 2) or a similar commercially available botulinum vaccine (CSL Botulinum Vaccine Batch No 0708 15102) failed to produce sustained SN titres to *Cl. botulinum* C or D during the same period of

10 observation. Only when the Tasgel[®] vaccine (Composition 2) was administered in two injections five weeks apart was a measurable SN response obtained, even so the titres were not sustained beyond weeks 26 (unlike Composition 1). Thus the SN titres indicate that long term protection (12 months) associated with measurable SN titres, can be achieved in cattle vaccinated with a single dose of the botulinum C and D

15 vaccine (Composition 1), whereas two doses of the other vaccines tested are required to achieve such titres.

A specific ELISA was used to monitor total antibody levels to *Cl. botulinum* following vaccination. Although this test does not discriminate between C and D

20 serotypes, it is particularly useful in monitoring antibody levels below the sensitivity of the SN test. The mean ELISA titres are presented below in Table VI as OD_{405nm} for 1/100 dilutions of sera from each of the experimental groups.

Table VI: Mean ELISA titres

25

Vaccine	Mean ELISA Titres (OD _{405nm} at 1/100 dilution)							
	Week							
	0	5	7	12	18	26	39	52
(Control)	0.063	0.065	0.063	0.075	0.078	0.111	0.190	0.140
Composition 1	0.054	0.901	1.071	1.393	1.555	1.648	1.577	0.517
Composition 2	0.081	0.530	0.461	0.386	0.235	0.284	NT	NT
Composition 2	0.086	0.510	1.299	1.177	1.114	0.773	0.523	0.288
CSL Botulinum Vaccine	0.037	0.745	0.666	0.722	0.674	0.435	NT	NT

NT = not tested

- 10 -

ELISA titres were detected in all groups at week 5. The titres from the single dose botulinum vaccine (Composition 1) continued to increase over a 40 week period, in contrast to the other single dose vaccine groups, where ELISA titres had rapidly declined by week 26. These results demonstrate that the single dose botulinum vaccine according to Composition 1 provides a sustained and elevated immune response to cattle without causing unacceptable vaccine site reactions.

EXAMPLE 2: EVALUATION OF SINGLE DOSE BOTULINUM VACCINE IN PREGNANT ANIMALS AND CALVES

Composition 1 was assessed in 5 to 12 month old calves and 2 to 8 year old pregnant cows which had not been previously treated with botulinum C or D vaccines. *Bos indicus* Brahman cattle were vaccinated with either a single 2mL dose, a single 4mL dose and two 2mL doses, the second being administered 14 days after the initial vaccination. Vaccine compositions were prepared and administered according to Table VII.

Table VII: Vaccination regime

Vaccine/Adjuvant	Dose Vol. (mL)	Vaccination (Week)	Animal administered to
Control	-	-	Calves
Composition 1	2	0	Calves
Composition 1	4	0	Calves
Composition 1	2	0,2	Calves
Control	-	-	Pregnant Cows
Composition 1	2	0	Pregnant Cows
Composition 1	4	0	Pregnant Cows
Composition 1	2	0,2	Pregnant Cows

The rectal temperatures, site reactions and general well being of the cattle was monitored for eight weeks. No significant differences between the vaccinate and non-vaccinate cattle were observed at the end of the trial. The cattle remained in good health and were grazing well. Site reactions were difficult to detect on a daily basis

- 11 -

depending on the movement of the animals neck and head. Site reactions were hard with no "fluid feel". Swelling was confined to the dermis and subdermal. There was no evidence of pain when firmly pressed. Repeated vaccination indicated that the pregnant cattle were more sensitive than the calves, as site reactions were quite large, but as in the case of the first vaccination these site reactions were resolving, as observed by the decreasing site reaction size. Importantly all site reactions were resolving by the end of the trial, at Week 8.

Eight weeks after the first vaccination site reactions on selected cattle were excised and the area beneath the skin examined for necrosis, inflammation, cellular infiltration and unadsorbed vaccine. The histopathology of the excised vaccination sites showed post-vaccinal subcutaneous granulomatous nodules up to 30mm in depth. Macrophages comprised 60 to 80% of cell mass. The dermis was relatively free of inflammatory change. In some cases the nodule extended into the underlying skeletal muscle. This suggests that the injection may have been more than just subcutaneous and probably reflects variable inoculation techniques. Oily adjuvant was dispersed within phagocytic vesicles within macrophages and in some cases remained in the tissue. There was no widespread necrosis within the granuloma. The lymphoproliferation within the granuloma was consistent with immunological stimulation. The presence of oily adjuvant was not unlike other reports on oil emulsion vaccines (e.g. J. Bollinger (1970) J.Pharm. Sci. 59 (8), 1084-1088).

The cattle were bled at Weeks 0 and 8, serum samples prepared and Botulinum C and D SN's determined by the mouse SN assay. Botulinum antibody levels were also determined by ELISA. The results indicated an immune response. The bivalent Botulinum C & D oil emulsion vaccine was shown to be safe in *Bos indicus* calves and pregnant cows. Although vaccination produced initial site reactions they had resolved by the conclusion of the trial. There was no pain associated with the swelling, and negligible tissue damage occurred. Excised site reactions indicated a typical immune response with localised lymphocyte proliferation. Systemic effects, following vaccination were negligible.

EXAMPLE 3: THE PREPARATION AND EVALUATION OF A SINGLE DOSE LEPTOSPIRA VACCINE

The safety and efficacy of single and two dose *Leptospira hardjo* vaccines formulated with the non-mineral oil ISA207 (Composition 4) was compared with

- 12 -

the standard two dose vaccine formulated with Tasgel[®] (Composition 3) and to a two dose vaccine formulated with potassium aluminium sulphate (Pot Alum; Composition 5). The animal group vaccinated with Composition 3 is referred to as Group B; those vaccinated with one and two dose of Composition 4 are referred to as Groups C and D respectively and the group vaccinated with Composition 5 are referred to as Group E. Vaccine compositions were prepared according to Table VIII below. A 2×10^9 organisms/2mL dose of *Leptospira hardjo* antigen at a concentration of 1.08×10^{10} organisms/ml was used in all vaccines.

10 **Table VIII: Leptospira Vaccine Composition**

Components	Composition (Amount % v/v)		
	3	4	5
<i>Leptospira hardjo</i>	9.26	9.26	9.26
Thiomersal	1	1	1
Normal Saline	59.74	34.74	58.44
Tasgel [®]	30	-	-
ISA207 [®]	-	55	-
Pot alum (10% w/v)	-	-	25.12
sodium hydroxide (10% w/v)	-	-	6.18

Cattle (approximately 6 months old) were vaccinated by subcutaneous injection and the rectal temperatures, site reactions and weight of the calves monitored.

15 Groups B, C and E were vaccinated a second time at Week 4. No general changes in behaviour or well being of the cattle were observed. Site reactions were estimated by palpation. A summary is shown below in Table IX, wherein the results are given as group mean site reaction (cm³) and number of cattle with a site reaction /number of cattle per group at each time point for each injection.

Table IX: Mean Site Reaction Volume in Cattle

Animal Group	Composition	Vaccine/Adjuvant	Mean Site Reactions (cm ³)					
			WEEK					
			2	4	6		10	
					1° ^a	2°	1°	2°
A	-	Control	-	-	-	-	-	-
B	3	<i>L.hardjo</i> /Tasgel [®]	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
C	4	<i>L.hardjo</i> /ISA207	11.2 (4/10)	10.4 (3/8)	5.2 (2/8)	0.4 (1/8)	1.6 (1/8)	0 (0/8)
D	4	<i>L.hardjo</i> /ISA207	8 (3/10)	4.4 (3/10)	3.2 (3/9)	-	1.4 (2/10)	-
E	5	<i>L.hardjo</i> /Pot Alum	0 (0/10)	0 (0/9)	0 (0/9)	0 (0/9)	0 (0/10)	0 (0/10)

a. 1° = vaccination site 1 - RHS of neck

2° = vaccination site 2 - LHS of neck

- 5 Detectable site reactions were observed with the oil adjuvanted vaccines but were generally considered to be minor and all had resolved by week 10. Two calves had large site reactions, one each from Group C and Group D at Week 4, but these had almost resolved by Week 10. A *L. hardjo* ELISA as described below and a Microscopic Agglutination Test (MAT) assay (according to Australian Techniques
- 10 Manual Standard Diagnostics) were carried out on serum samples collected from the blood of the cattle. The group mean *L. hardjo* ELISA results are presented below in Table X and the group mean MAT results are presented below in Table XI.

- 15 *L. hardjo* ELISAs were performed on individual serum samples. *L. hardjo* antigens were prepared by sonication of 5×10^7 cells/ml in PBS (pH7.3) for 40 seconds at 2-8°C. 100 µl antigen was added to 96 well flat-bottomed microtitre plates which were then incubated overnight at 4°C after which unbound antigens were removed by washing the plates with phosphate buffered saline PBS/Tween 20 (0.1% v/v; pH7.2). 200 µl Blocking buffer (Bovine serum albumin; 1% w/v in PBS/Tween 20) was then
- 20 added and the plates incubated for 60 minutes at room temperature after which they were washed with PBS/Tween 20. Dilutions of cattle sera (and positive and negative sera controls) were added to the wells and the plates incubated for 60 minutes at room temperature. The plates were washed with PBS/Tween 20. A dilution of rabbit anti-bovine horseradish peroxidase conjugate (NORDIC) in PBS/Tween 20 was added and

- 14 -

- the plates incubated for a further 60 minutes at 37°C and then washed with PBS/Tween 20. Activated substrate o-phenylenediamine dihydrochloride dissolved in citrate phosphate buffer (pH4.2) at 0.34mg/ml and activated by the addition of 0.3% hydrogen peroxide was added to the plate. The reaction was stopped by the addition of 8N sulphuric acid after 30 min at room temperature and absorbance at 492 nm was read using a Titertek Multiscan reader. Results are shown in Table X.

Table X: *Leptospira hardjo* ELISA (Group Mean ELISA Units)

Animal Group	Week			
	0	4	6	10
A	<1	<1	1	<1
B	<1	<1	36	22
C	<1	7	144	206
D	<1 ^a	16	18	76
E	<1 ^b	<1	40	24

- 10 a. One calf had a titre of 20
b. One calf had a titre of 5

- The standard vaccine in Group B at Week 4 did not generate detectable titres, while the test vaccines incorporating ISA207 (Groups C and D) did induce antibody formation by this time. Following a second dose the titres of Group C animals (ISA207 two dose) were 4 times higher than those induced by the standard vaccine. By week 10, both vaccines incorporating ISA207 (Group C two dose and Group D single dose) generated titres 6 and 3 times higher respectively than the standard vaccine. The use of pot alum to replace Tasgel® (Group E) resulted in similar titres to the standard vaccine. A statistical analysis of Week 6 and Week 10 data shows a significant difference ($p < 0.05$) between the vaccines incorporating ISA207 and the standard vaccine.

Table XI - *Leptospira hardjo* MAT (Group Mean Titres)

Animal Group	Week	
	6	10
A	3.1	3.1
B	18.9	10.9
C	25.0	200.0
D	19.8	34.0
E	Not tested	Not tested

- 15 -

The MAT analysis at Week 6 reveals a group mean titre for groups B to D of approximately 18.9 to 25 compared to the non-vaccinated control (Group A).

- 5 However, there was no significant difference between vaccine groups ($p > 0.05$). A MAT of 25 is required for a detectable *L.hardjo* response, (seroconversion or natural infection). The MAT analysis at Week 10 indicates larger differences between the groups, with a significant difference between groups B and C ($p < 0.05$).

The vaccine formulated with ISA207 was demonstrated to be safe in cattle.

- 10 Temperature rises were minimal following injection and no adverse clinical effects or body weight effects were observed.

- 15 The vaccine formulated with ISA207 induced significant antibody responses as measured both by ELISA and MAT. A response was detectable by ELISA at week 4 after only one dose of ISA207 vaccines. A single dose of ISA207 vaccine generated titres at week 6 which were comparable with those generated following two doses of aluminium adjuvanted vaccine. By week 10 the titres of double dosed aluminium adjuvanted vaccine groups had declined, while the single dosed ISA207 group titres continued to increase, suggesting the likelihood of an enhanced duration of immunity.
- 20 Two doses of the ISA207 vaccine generated extremely high titres, more typical of levels induced by disease rather than conventional vaccination. These titres were still increasing at the scheduled completion of the trial (week 10 post vaccination).

25 **EXAMPLE 4: THE PREPARATION AND EVALUATION OF
MULTIVALENT SINGLE DOSE VACCINES**

- 30 Multivalent vaccine compositions containing antigens against a number of diseases and vaccine compositions containing a number of different adjuvants were prepared according to Table XII. Volumes of antigens used in the compositions depended on the concentrations of the bulks from which they are taken.

-16-

Table XII: Multivalent Vaccine Compositions

Component	Composition (amount % v/v)													
	6	7	8	9	10	11	12	13	14					
<i>Cl. perfringens</i> D toxoid	2.6	5.2	1.44	5.20	2.35	2.35	2.88	2.88	2.0					
<i>Cl. novyi</i> B toxoid	3.0	5.9	0.4	5.74	0.8	0.8	0.8	0.8	1.4					
<i>Cl. chauvoei</i> anaculture	2.2	4.4	1.62	4.40	2.86	2.33	3.25	3.25	3.26					
<i>Cl. septicum</i> toxoid	0.7	1.4	1.33	1.4	2.74	2.74	2.67	2.67	1.12					
<i>Cl. tetani</i> toxoid	4.5	9	0.14	9	0.28	0.28	0.28	0.28	0.28					
<i>C. pseudotuberculosis</i>	-	-	0.15	0.84	0.30	0.30	0.30	0.30	0.24					
Normal Saline	-	-	63.92	-	34.5	30.0	14.83	9.83	36.2					
MilliQ	56.0	18.1	-	22.2	-	-	-	-	-					
Thiomersal	1	1	1	1	1	1	1	1	1					
M207	-	55.0	-	50	54	54.4	54	54	55					
Tasgel	30	-	30	-	-	-	-	-	-					
Quil A	-	-	-	-	1.18	1.18	-	-	-					
DEAE Dextran	-	-	-	-	-	5	-	-	-					
Dextran Sulphate	-	-	-	-	-	-	20	-	-					
DDA	-	-	-	-	-	-	-	25	-					
MDP	-	-	-	-	-	-	-	-	0.5					

- 17 -

- 5-in-1 compositions adjuvanted by Tasgel® (Composition 6) and M207 (Composition 7) were prepared; the five antigens included are described in Table XII. Analogous 6-in-1 compositions adjuvanted by Tasgel® (Composition 8) and M207 (Composition 9) were also prepared by additionally including
- 5 *Corynebacterium pseudotuberculosis*. Compositions containing various supplements or co-adjuvants were also prepared as detailed in Table XII (Compositions 10 to 14).

- The vaccines were assessed in the rabbit potency and *Cl chauvoei* guinea pig challenge tests according to the Therapeutics Goods Order No. 30 (Australian Government Publishing Services), amended where necessary. Mice serum neutralisation titres of pooled group sera for *Cl. novyi*, *Cl. tetani*, *Cl. septicum* and *Cl. perfringens* D were carried out according to the Therapeutic Goods Order No. 30 (Australian Government Publishing Services, 1987). CLA serum neutralisation titres for individuals and pooled sera samples based on observations by Muckel & Giles,
- 15 Am. J. Vet. Res. 44, 1149-1153, 1983 in order to measure a response to the *Corynebacterium pseudotuberculosis* antigens. The *Cl chauvoei* ELISA was based on the method described in Crichton, Solomon and Barton, Biologicals 18, 49-54, 1990. The results of these tests are detailed below in Table XIII

20 **Table XIII: Effectiveness of multivalent vaccines**

Composition	Rabbit Potency SN (U/mL)					<i>Cl. chauvoei</i>	
	<i>Cl. tetani</i>	<i>Cl. perfringens</i> D	<i>Cl. septicum</i>	<i>Cl. novyi</i> B	CLA	ELISA (U/mL)	Guinea Pig Challenge
6	2.9	9-13.5	5.6-8.4	4.7-7.1	<1	118	8/9
7	6.2-9.3	13.5-20.2	3.8	>10.67	<1	262	6/10
8	NT	NT	NT	NT	2.5	112	4/5
9	>12.66	>27.8	2.5-3.8	>13	1.1	230	NT
10	8.4-12.7	18.6-27.8	8.4-12.7	>10.7	<1	108	5/5
11	>12.7	8.2-12.4	8.4-12.7	7.1-10.7	<1	77	NT
12	NT	NT	NT	NT	<1	>200	4/5
13	NT	NT	NT	NT	<1	166	5/5
14	>12.9	>28	9.5-14.2	>12	2.7	172	10/10
CODEX PASS	2.5	5	2.5	3.5	1.5	60	10/10

- 18 -

The M207 adjuvanted 5-in-1 and 6-in-1 vaccines (Compositions 7, 9, 10, 11, 12, 13, and 14) passed potency tests for the four clostridial toxoid fractions (*Cl. tetani*, *Cl. perfringens* D, *Cl. septicum* and *Cl. novyi* B following a single dose vaccination.

- 5 This result is similar to that of the Tasgel® vaccine (Composition 8) after two doses. The addition of co-adjuvants was found to improve the responses to *Cl. chauvoei* and/or *Corynebacterium pseudotuberculosis*.

- 10 Several vaccines were assessed in fine wool Merryville Merino lambs for safety and efficacy. Site reactions were not found on any lambs vaccinated and no adverse effects on their general well being was observed.

- Blood was collected at several time intervals after initial vaccination and at four weeks post annual vaccination. Serum samples were collected from blood
15 centrifuged for 15 minutes at 3000 RPM at room temperature. The following assays were performed on serum samples:

- Cl. perfringens* D ELISA were performed on individual serum samples using purified rabbit anti-*Cl. perfringens* epsilon toxin diluted in carbonate buffer (pH 9.6) in a 96
20 well microtitre plate. This was incubated at 37°C for 2 hours before purified *Cl. perfringens* toxin diluted in phosphate buffered saline/Tween 20 (0.1% w/v; (pH7.2) was added and the plate incubated at 37°C for 1 hour. Dilutions of sheep sera (and positive and negative sera) were added to the wells and the plate incubated for 1 hour at 37°C. The plates were then washed with PBS/Tween and a dilution of rabbit anti-
25 sheep IgG horseradish peroxidase conjugate (Biorad) in PBS/Tween added. The plate was incubated at 37°C for a further hour and then washed with PBS/Tween. Activated substrate (2,2 azino-di-3-ethylbenzthiazolinosulfonate) dissolved in citrate phosphate buffer (pH 4.2) at 1 mg/ml and activated by addition of 0.3% hydrogen peroxide was added to the plate. Absorbance at 405 nm was read after 30 to 60
30 minutes using a Titertek Multiscan reader. A similar procedure was carried out for *Cl. tetani* using purified *Cl. tetani* toxoid on individual serum samples. The results of these tests are detailed below in Tables XIV and XV:

- 19 -

Table XIV: *Cl. perfringens* D ELISA Titre (U/mL)

Composition	Vaccine/ Adjuvant	<i>Cl. perfringens</i> D ELISA Titre U/mL							
		Marking	Wk 4	Wk 8	Wk 12	Wk 19	Wk 26	Wk 51	Wk 55
	Non-vaccinate	1.6	<1	<1	<1	<1	<1	<1	<1
6	5in1/ Tasgel®	2.2	<1	<1	2.9	<1	<1	<1	1.4
6 ^a	5in1+Se/ Tasgel®	3.6	<1	<1	1	<1	<1	<1	2.4
7	5in1/M207	<1	2.3	2.5	9.2	14	7.6	7	11.9
7 ^a	5in1+Se/ M207	5	1.7	1	4.6	6	2.3	4.4	29.6
9	6in1/M207	2.6	10.8	5.5	12	24	8.8	NT	NT
9 ^a	6in1+Se/ M207	2.8	3.2	1.8	5.6	11.2	1.4	NT	NT
10	6in1/M207 Quil A	1.2	7.2	5.6	9.8	4.4	2.8	NT	NT

a. Selenium (Se) added as sodium selenate as a supplement at a level of 1mg/mL of Selenium

5 Table XV: *Cl. tetani* ELISA Titre (U/mL)

Composition	Vaccine/ Adjuvant	<i>Cl. tetani</i> ELISA Titre (U/mL)							
		Marking	Week 4	Week 8	Week 12	Week 19	Week 26	Week 51	Week 55
	Non-Vaccinate	1.9	<1	<1	<1	<1	<1	<1	<1
6	5in1/ Tasgel®	<1	<1	<1	<1	<1	<1	<1	1.9
6 ^a	5in1+Se/ Tasgel®	<1	<1	<1	<1	<1	<1	<1	1.6
7	5in1/M207	<1	<1	<1	2.6	<1	1	<1	11
7 ^a	5in1+Se/ M207	1.4	<1	1.1	<1	<1	<1	<1	10.4
9	6in1/M207	<1	<1	2.2	1.4	1	1.3	NT	NT
9 ^a	6in1+Se/ M207	1.5	1.2	2.7	4.4	1	1.3	NT	NT
10	6in1/M207 -QuilA	<1	1.5	2.1	3.2	<1	<1	NT	NT

a. Selenium (Se) added as sodium selenate as a supplement at a level of 1mg/mL of Selenium

- 20 -

The 6-in-1/M207-Quil A vaccine composition was assessed in randomly selected lambs. Pooled group sera was shown to have maternal antibodies as detected by *Cl. perfringens* and *Cl. tetani* ELISAs. The vaccines formulated with M207 and also with co-adjuvant Quil A gave similar responses in both the *Cl. perfringens* and *Cl.*
5 *tetani* ELISAs. These responses were higher than that observed with the Tasgel® vaccines (Composition 6). The addition of selenium as a mineral supplement did not appear to influence the responses to the antigens. All M207 formulations gave variable *Cl. tetani* ELISA titre responses regardless of the maternal antibody.

- 21 -

CLAIMS

1. A vaccine composition which is an injectable multi-phase emulsion of the water in oil in water type having a viscosity of 100mPas or less, comprising:
 - a) an oily adjuvant acceptable for veterinary purposes comprising:
 - i) a synthetic hydrocarbon being between 40% and 60% by weight of the emulsion and
 - ii) an emulsifier selected so that the inversion point of the resulting emulsion is between 25°C and 45°C and being between 2% and 10% by weight of the emulsion and
 - b) an aqueous phase comprising one or more antigens selected from diseases of cattle and sheep.
2. A vaccine composition as claimed in Claim 1 wherein the synthetic hydrocarbon is liquid at 4°C.
3. A vaccine composition as claimed in any one of Claim 1 or Claim 2 wherein the synthetic hydrocarbon is a polyisobutene or a polyisoprene.
4. A vaccine composition as claimed in Claim 3 wherein the synthetic hydrocarbon is a polyisobutylene.
5. A vaccine composition as claimed in any one of Claim 1 to 4 wherein the emulsifier is a fatty acid ester or ether of a sugar or polyol or a derivative thereof.
6. A vaccine composition as claimed in Claim 5 wherein the emulsifier is an ester or ether of a fatty acid having 8 to 22 carbons atoms in the fatty acid chain.
7. A vaccine composition as claimed in Claim 5 or Claim 6 wherein the emulsifier is an ester or ether of oleic, stearic or ricinoleic acid or is mannide oleate or a PEG oleate.
8. A vaccine composition as claimed in any one of claims 1 to 7 further comprising a co-adjuvant.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 97/00871

A. CLASSIFICATION OF SUBJECT MATTER																						
Int Cl ⁶ : A61K 9/113, 39/02, 39/05, 39/08, 39/116																						
According to International Patent Classification (IPC) or to both national classification and IPC																						
B. FIELDS SEARCHED																						
Minimum documentation searched (classification system followed by classification symbols) A61K 9/113, A61K 39/- and keywords as below																						
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched AU: IPC as above																						
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) DERWENT, CHEMICAL ABSTRACTS: (HYDROCARBON: or POLYISOBUTENE: or POLYISOPRENE: or POLYISOBUTYLENE or ISA207) or (CLOSTRID: or BOTUL: or LEPTOSPIRA or CORYMEBACTER:) and VACCINE: and EMULS:																						
C. DOCUMENTS CONSIDERED TO BE RELEVANT																						
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																				
X	AU 59610/90 (643690) B (SOCIETE D'EXPLOITATION DE PRODUITS POUR LES INDUSTRIES CHIMIQUES (S.E.P.P.I.C)) 10 January 1991 Whole document	1-8																				
X	WO 95/25542 A (SOCIETE D'EXPLOITATION DE PRODUITS POUR L'INDUSTRIE CHIMIQUE S.E.P.P.I.C) 28 September 1995 Whole document	1-8																				
P.X	EP 781559 A (JURIDICAL FOUNDATION, THE CHEMO-SERO-THERAPEUTIC RESEARCH INSTITUTE KUMAMOTO -KEN) 2 July 1997 Whole document	1-8																				
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex																						
<p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A"</td> <td>document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T"</td> <td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E"</td> <td>earlier document but published on or after the international filing date</td> <td>"X"</td> <td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L"</td> <td>document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y"</td> <td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O"</td> <td>document referring to an oral disclosure, use, exhibition or other means</td> <td>"&"</td> <td>document member of the same patent family</td> </tr> <tr> <td>"P"</td> <td>document published prior to the international filing date but later than the priority date claimed</td> <td></td> <td></td> </tr> </table>			"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E"	earlier document but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family	"P"	document published prior to the international filing date but later than the priority date claimed		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention																			
"E"	earlier document but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone																			
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art																			
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family																			
"P"	document published prior to the international filing date but later than the priority date claimed																					
Date of the actual completion of the international search 18 February 1998		Date of mailing of the international search report 3 MAR 1998																				
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929		Authorized officer R.L. POOLEY Telephone No.: (02) 6283 2242																				

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 97/00871

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AU 59630/90 (655155) B (SOCIETE D'EXPLOITATION DE PRODUITS POUR LES INDUSTRIES CHIMIQUES (S.E.P.P.C)) 10 January 1991 Whole document	1-8
Y	WO 93/00160 A (EMORY UNIVERSITY) 7 January 1993 Whole document	1-8
A	Patent Abstracts of Japan, JP 8-027028 A (NIPPON SEIBUTSU KAGAKU KENKYUSHO) 30 January 1996	1-8
A	EP 278103 A (AMERICAN CYANAMID COMPANY) 17 August 1988	1-8
A	US 3579633 A (THOMSON) 18 May 1971	1-8
A	GB 1128325 A (THE WELLCOME FOUNDATION LIMITED) 25 September 1968	1-8

Information on patent family members

PCT/AU 97/00871

particulars which are merely given for the purpose of information.

END OF ANNEX